

## CLAIMS

1. A method for determining the concentration of a substrate in a sample solution using a biosensor comprising an electrically insulating base plate, an electrode system having a working electrode, a counter electrode and a third electrode to be used as an interfering substance detecting electrode, each being formed on said base plate, and a reaction layer which contains at least an oxidoreductase and an electron mediator and is formed on the electrode system omitting the third electrode, wherein said electron mediator is reduced by the produced electrons upon reaction between the substrate contained in the sample solution and the oxidoreductase to measure a reduced amount of said electron mediator electrochemically,

said method being characterized by comprising:

(a) a step of applying a voltage between the counter electrode and the third electrode;

(b) a step of supplying the sample solution to the reaction layer;

(c) a step of detecting an electrical change between the counter electrode and the third electrode due to supply of the sample solution to the reaction layer;

(d) a step of measuring a current flowing between the counter electrode and the third electrode after said detecting step (c);

(e) a step of releasing voltage application between

the counter electrode and the third electrode after said measuring step (d);

(f) a step of applying a voltage between the working electrode and the counter electrode; and

(g) a step of subsequently measuring a current flowing between the counter electrode and the working electrode.

2. The method for determining substrate in accordance with claim 1, wherein said step (f) also applies a voltage between the working electrode and the third electrode.

3. A method for determining the concentration of a substrate in a sample solution using a biosensor comprising an electrically insulating base plate, an electrode system having a working electrode, a counter electrode and a third electrode to be used as an interfering substance detecting electrode, each being formed on said base plate, a reaction layer which contains at least an oxidoreductase and an electron mediator and is formed on said electrode system omitting the third electrode, and a cover member forming a sample solution supply pathway to introduce a sample solution from a sample solution supply port into said reaction layer on said base plate, said third electrode being located upstream of said sample solution supply pathway from said reaction layer, wherein said electron mediator is reduced by the produced electrons upon reaction between the substrate contained in the sample solution and

the oxidoreductase to measure a reduced amount of said electron mediator electrochemically,

said method being characterized by comprising:

(a) a step of applying a voltage between the counter electrode and the third electrode;

(b) a step of supplying the sample solution to the reaction layer;

(c) a step of detecting an electrical change between the counter electrode and the third electrode due to supply of the sample solution to the reaction layer;

(d) a step of measuring a current flowing between the counter electrode and the third electrode after said detecting step (c);

(e) a step of releasing voltage application between the counter electrode and the third electrode after said measuring step (d);

(f) a step of applying a voltage between the working electrode and the counter electrode; and

(g) a step of subsequently measuring a current flowing between the counter electrode and the working electrode.

4. The method for determining substrate in accordance with claim 3, wherein said step (f) also applies a voltage between the working electrode and the third electrode.

5. The method for determining substrate in accordance with claim 3, wherein a biosensor disposed with a layer

essentially composed of lecithin on an exposed surface of the sample solution supply pathway of said cover member is used.

6. The method for determining substrate in accordance with claim 1, wherein a biosensor further containing a hydrophilic polymer in said reaction layer is used.

7. The method for determining substrate in accordance with claim 3, wherein a biosensor further containing a hydrophilic polymer in said reaction layer is used.

FOR REF. 482200